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Volatile profile of Spanish-style green table olives prepared from different cultivars grown at different locations

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Running title: Volatile profile of Spanish-style green table olives

Abstract

The volatile profiles of Spanish-style green table olives elaborated with Manzanilla, Gordal and Hojiblanca cultivars grown at different locations in Spain were established by solid phase micro-extraction (SPME) and gas chromatography coupled to mass spectrometry (GC-MS). A total of 102 volatile compounds were identified, belonging to distinct chemical classes, and 20 of them are reported for the first time in table olives. The headspace profile was predominated by alcohols and phenols, followed by acids and esters, whereas the relative amounts of the remaining classes were quite lower (< 5% in general). The principal compounds characterizing the headspace for most samples were *p*-creosol, phenylethyl alcohol, acetic acid, ethanol, benzyl alcohol, ethyl acetate, and (Z)-3-hexen-1-ol. Significant differences in the proportions of volatile compounds between samples from the Gordal cultivar and those from Manzanilla and Hojiblanca cultivars were detected and statistically visualized by principal component analysis (PCA). Among all the identified compounds, only (E)-2-decenal showed significant differences between the three cultivars without being significantly affected by locations where the fruits were grown.

Keywords: table olives, volatile composition, olive cultivar, SPME, GC-MS, PCA

Highlights

- More than 100 headspace compounds were identified in Spanish-style green table olives.
- Headspace profile of product was predominated by alcohols and phenols.
- PCA discriminated samples according to olive cultivar.

1. Introduction

Spain is the main producer of table olives in the world, with 573,371 t in the season 2013/2014, and over 50% of its production corresponds to Spanish-style green table olives (ASEMESA, 2015). This type of table olive is considered the main fermented vegetable product in western countries. Its processing consists of a treatment with alkaline lye (1.8-2.5%, w/v NaOH) to hydrolyze the bitter glucoside oleuropein, followed by a washing step to remove the excess alkali. A solution of NaCl (10-13%, w/v) is then added, and a lactic acid fermentation takes place (Rejano, Montaño, Casado, Sánchez, & de Castro, 2010). After this step, which can last a few months, the fruits are kept in the fermenter until they are marketed either in bulk with their own fermenting brine or packed in small containers with an acidified cover brine. The unique and pleasant flavor of this product is probably the most appreciated characteristic for consumers. The flavor of table olives is closely related to both the qualitative and quantitative composition of volatile compounds and can be influenced by a number of factors, including olive cultivar, fruit ripeness stage, and processing method (Sabatini and Marsilio, 2008). Optimal processing conditions and microbial spoilage have been extensively studied for Spanish-style green table olives, yet the literature on the volatile composition of this product is rather limited. Previous studies regarding this subject were carried out to evaluate the major headspace compounds of olive brine (Montaño, Sánchez, & Rejano, 1990), to identify the volatile compound responsible of the unpleasant odor of zapatera olives (Montaño, de Castro, Rejano, & Sánchez, 1992), to screen for key odor compounds in Moroccan green table olives (Iraqi, Vermeulen, Benzekri, Bouseta, & Collin, 2005), to compare the volatile compounds in Spanish-style, Greek-style and Castelvetrano-style green olives (Sabatini and Marsilio, 2008),

and to evaluate the effects of regulated deficit irrigations on the profile of volatile compounds (Cano-Lamadrid et al., 2015).

Today, solid-phase microextraction (SPME) followed by gas chromatography coupled to mass spectrometry (GC-MS) is one of the most often used techniques for analysis of volatile compounds in foods (Merkle, Kleeberg, & Fritsche, 2015). SPME-GG-MS has been applied to study the volatile composition of raw olives as well as of different types of table olives. A total of 34 volatile compounds were identified in intact raw olives from three Portuguese olive cultivars (Cobrançosa, Madural, and Verdeal Transmontana), with the main contributors being (Z)-3-hexen-1-ol, hexanal, and (Z)-3-hexen-1-ol acetate (Malheiro, Casal, Cunha, Baptista, & Pereira, 2015). These authors demonstrated that volatile composition of olives is dependent on the olive cultivar, and is highly influenced by olives maturation. In unfermented "Campo Real" table olives, the main aroma compounds identified were ethanol, 2-butanol, 3-hexen-1-ol, ethyl hexanoate, benzaldehyde, eucalyptol, γ-terpinene, fenchone, linalool, and terpinen-4-ol (Navarro, de Lorenzo, & Pérez, 2004). In green Sicilian table olives from five different cultivars (Brandofino, Castriciana, Nocellara del belice, Passalunara, and Manzanilla), a total of 52 compounds were identified after 60 days of fermentation (Aponte et al., 2010). This study evidenced several differences in the volatile profiles among cultivars and considerable changes in their profiles during storage. In the Portuguese preparation known as "alcaparras" table olives, 42 volatile compounds consisting manly of aldehydes were identified (Malheiro, de Pinho, Casal, Bento, & Pereira, 2011). Again, it was demonstrated that the volatile profile was influenced by the olive cultivar used. In Greek-style green table olives, analyses of volatile compounds by SPME-GC-MS have been more numerous. Using olives from Nocellara del Belice cultivar, Martorana et al. (2015) identified 49 volatile compounds, with acids, alcohols and aldehydes being

detected at the highest concentrations. A more complex volatile profile (82 volatiles) was found with olives from Bella di Cerignola cultivar (De Angelis et al., 2015). A comparative study between Greek-style green table olives from Giarraffa and Grossa di Spagna cultivars was conducted by Randazzo et al. (2014). Notable differences among volatile compounds were detected (35 compounds in Giarraffa samples vs. 24 in Grossa di Spagna ones), indicating that cultivar strongly influenced the final product. Another comparative study was carried by Bleve et al. (2015) in Greek-style black table olives from Conservolea and Kalamàta cultivars. Forty-six compounds were identified and principal component analysis (PCA) was carried out at three different fermentation times. Aldehydes were closely associated with the first stage of fermentation (30 days), isoamylalcohols and styrene with the middle stage (30-90 days) and ethyl esters and fatty acids with the final stage (180 days). Finally, in Spanish-style table olives from Manzanilla cultivar, a total of 43 volatile compounds have been identified (Cano-Lamadrid et al., 2015). The five most abundant volatile compounds by these authors were: acetic acid, 2-decenal, tetrahydrogeraniol, 1,4-dimethoxybenzene, and 4,8-dimethyl-1,3,7-nonatriene. The main objective of the present work was to comparatively study the volatile profile of Spanish-style green table olives produced from the cultivars Manzanilla, Gordal, and Hojiblanca using the HS-SPME-GC-MS technique. These three cultivars are the most prominent cultivars dedicated to table olives in Spain (Hojiblanca, 51% of the total exports in 2014; Manzanilla, 33%; and Gordal, 8%) (ASEMESA, 2015). Manzanilla olive is a fleshy olive with a fine texture, spherical shape and medium size. Gordal olive has a very low oil content and is larger than most. Hojiblanca variety is a dual-purpose olive, that is, it can be used either for making oil or for table olives. In order to choose the most adequate HS-SPME

procedure based on extraction efficiency, different sample preparation procedures were previously assessed.

2. Materials and Methods

2.1. Samples and chemicals

Manzanilla, Gordal, and Hojiblanca cultivars, grown at different locations with ample tradition in Spanish-style table olives processing, were selected. The growing locations were: *Manzanilla cv*: Alcalá de Guadaira (Seville), Posadas (Córdoba), and Almendralejo (Badajoz), the corresponding samples were denoted by the codes MAl, MC, and MAm, respectively; *Gordal cv*.: Utrera (Seville) and Arahal (Seville), the corresponding samples were denoted by the codes GU and GA, respectively; and *Hojiblanca cv*.: Alameda (Málaga), Estepa (Seville), and Casariche (Seville), with the corresponding samples being denoted by the codes HA, HE, and HC, respectively. The olives were harvested between September 23rd and October 26th, 2013, at their mature-green stage and transported to our laboratories for processing. At the laboratory, the olives were placed in polyethylene vessels (5.2 kg fruits plus 3.4 L liquid capacity) and the typical steps of Spanish-style method were carried out. An alkaline treatment was carried out using a lye solution of 1.90-2.10% w/v NaOH. The olives remained in this solution until the lye had penetrated two-thirds of the way through the flesh. Then, a long-period water washing (11-17 h duration) was applied. The only exception was the sample HE, which was subjected to two washings of 1h and 1.5 h. This change in washing stage was decided in view of the rapid evolution of alkaline treatment, in order to prevent possible damage of fruits due to the effect of NaOH on texture and, at the same time, an excessive loss of sugars, which would affect the lactic acid fermentation.

Finally, the olives were covered with brine (11.4% NaCl) and kept at room temperature for fermentation. The experiments were conducted in duplicate (denoted with the numbers 1 and 2 after each sample code) except for sample HC which was processed only once (due to the supplied amount of olives was not sufficient to make duplicate elaborations). Corrections to prevent any microbial spoilage were not necessary in the case of samples from Manzanilla or Gordal cultivars, but lactic acid was added to samples from the Hojiblanca cultivar at the end of fermentation in order to reach final pH values lower than 4 units. After 5 months of brining, once olives were totally fermented as indicated by the absence of reducing sugars according to the Fehling´s test, sampling was performed for the determination of chemical and microbiological characteristics, and analysis of volatile compounds.

Isopropyl alcohol, ethanol, 2-butanol, 1-propanol, isobutanol, 1-butanol, isopentanol, 3-methyl-3-buten-1-ol, 1-pentanol, 3-methyl-2-buten-1-ol, 3-methyl-1-pentanol, 1-hexanol, (Z)-3-hexen-1-ol, 1-octen-3-ol, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, 1-nonanol, benzyl alcohol, phenylethyl alcohol, ethyl acetate, methyl propanoate, propyl acetate, methyl butanoate, methyl 2-methylbutanoate, isobutyl acetate, methyl 3-methylbutanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, isoamyl acetate, methyl hexanoate, ethyl hexanoate, hexyl acetate, ethyl (E)-3-hexenoate, methyl lactate, ethyl lactate, methyl octanoate, ethyl octanoate, methyl decanoate, ethyl decanoate, ethyl benzoate, benzyl acetate, methyl salicylate, ethyl salicylate, propanoic acid, isobutanoic acid, butanoic acid, 2-methylbutanoic acid, hexanoic acid, heptanoic acid, (E)-3-hexenoic acid, octanoic acid, decanoic acid, benzoic acid, pentanal, heptanal, octanal, 2-heptenal, nonanal, (E)-2-octenal, benzaldehyde, (E)- 2-decenal, limonene, 6-methyl-5-hepten-2-one, linalool oxide, linalool, α-terpineol, β-damascenone, geraniol, p-creosol, phenol, p-ethyl guaiacol, p-

cresol, p-propyl guaiacol, eugenol, 4-ethyl phenol, vanillin, tyrosol, octane, decane, o-xylene, styrene, dimethyl sulfide, theaspirane (mixture of theaspirane A and theaspirane B), dimethyl sulfoxide, butyrolactone, 1,4-dimethoxybenzene, and n-alkane standards (C7-C30) were supplied by Sigma-Aldrich (St. Louis, MO). Methyl (E)-3-hexenoate was purchased from Across Organics (Thermo Fisher Scientific, Madrid, Spain). Isoamyl lactate, methyl hydrocinnamate, and β-caryophyllene were purchased from TCI Chemicals (Cymit Química SL, Barcelona, Spain). Acetic acid was purchased from Panreac (Barcelona, Spain). Ultra-pure water from Milli-Q system (Millipore, Bedford, MA) was used throughout. De Man, Rogosa, Sharpe (MRS) agar and oxytetracycline-glucose-yeast extract (OGYE) agar were from Oxoid (Basingstoke, UK). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers.

2.2. Selection of sample preparation procedure for HS-SPME analysis

In order to assess the effect of the sample preparation procedure, three different sample preparations were tested: (1) the extraction of 5 g of homogenized olive pulp plus 5 mL of ultra-pure Milli-Q water, (2) the extraction of 5 g of homogenized olive pulp plus 5 mL of 30% (w/v) NaCl, and (3) the extraction of a 10 g aliquot of a homogenized sample obtained by mixing 20 g of pulp with 20 mL of a solution containing 30% (w/v) NaCl, 0.3% (w/v) ascorbic acid and 0.3% (w/v) citric acid. In all cases, the experimental conditions were adjusted so that the same amount of pulp was extracted. To assess the effect of sample dilution, the preparation mode 1 (dilution 1:1) was compared with (a) the extraction of 3.5 g of homogenized pulp plus 7 mL of ultra-pure Milli-Q water (dilution 1:2), and (b) the extraction of 2.5 g of homogenized pulp plus 7.5 mL of ultra-pure Milli-Q water (dilution 1:3). Three replicates per sample were

prepared and analyzed. All measurements were performed under constant stirring (600 rpm) using the following extraction conditions: equilibration time, 30 min; extraction temperature, 60 ºC; and extraction time, 30 min. The volume of the sample phase (10 mL) in the 15 mL vial was kept constant in all assays. This minimizes the headspace volume and improves extraction efficiency according to the operating instructions for SPME sampling supplied by Supelco. The experiments were carried out with Spanish-style green olives from the Manzanilla cultivar.

2.3. HS-SPME-GC-MS analyses

A 1 cm, 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) StableFlex fiber (Supelco, Bellefonte, PA) was used. This triple fiber was chosen in the present work in order to obtain the highest recoveries and a wider profile, according to previous studies from the literature for samples of fermented table olives (Aponte et al., 2010) or other fermented foods (Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, &López-Tamames, 2014). It combines the absorption properties of the liquid polymer with the adsorption properties of porous particles and has bipolar properties. Before the first use, the fiber was conditioned at 270 ºC for 1 h according to the supplier´s instructions. Olives (approximately 200 g), which were separated from brine and dried with a tissue, were pitted and then homogenized in a blender. Aliquots of 2.5 g of homogenized olive pulp were placed in a 15 mL glass vial, and 7.5 mL of 30% (w/v) NaCl were added. After the addition of a stirring bar (cross shaped PTFE bar of 5 mm long and 10 mm diameter, for stirring at 600 rpm), the vial was closed and placed in a water bath adjusted to 60 ºC. We used this relatively high temperature in order to improve extraction of semi-volatile compounds, as reported in other foods with high fat content such as cocoa products (Ducki, Miralles-García, Zumbé, Tornero, & Storey,

2008). After this step, fiber was manually inserted into the sample vial headspace during 60 min. After completion of the extraction process, the fiber was retracted prior to removal from the sample vial and immediately inserted into the injection port of the GC for desorption at 250 ºC for 15 min. All measurements were made in triplicate using different vials.

All GC-MS analyses were performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector and GC/MSD ChemStation software (version E.02.01.1177) (Agilent Technologies, Santa Clara, CA). A VF-WAX MS capillary column (30 m x 0.25 mm x 0.25 μm film thickness) from Agilent was used. The GC/MS conditions used were slightly modified from those described by Aprea et al. (2012). The injector port (equipped with a glass liner of 0.75 mm I.D.) was heated to 250 ºC. The injections were performed in the splitless mode. The carrier gas was helium at a constant flow of 1 mL min^{-1}. The initial oven temperature was 40 ºC (5 min), which was ramped up at 3 ºC/min to 195 ºC, and then at 10 ºC/min to 240 ºC and held there for 15 min. For the mass selective detector conditions, the quadrupole, ion source and transfer line temperatures were maintained at 150, 230, and 250 ºC, respectively. Electron ionization mass spectra in the full-scan mode were recorded at 70 eV in the range 40-400 amu. Peaks were identified by comparing their mass spectra, retention times and linear retention indices (RI) against those obtained from authentic standards. The compounds for which it was not possible to find authentic standards were tentatively identified by comparing their mass spectra with spectral data from the NIST 08 MS library as well as retention indices sourced from NIST Standard Reference Database. For the determination of the RI, a C7-C30 *n*-alkanes series was used, and the values were compared, when available, with values reported in the literature for similar chromatographic columns. The GC peak area of each compound was obtained from the

242 ion extraction chromatogram (IEC) by selecting target ions for each one. These ions
243 corresponded to base ion (*m/z* 100% intensity), molecular ion (M^+) or another
244 characteristic ion for each molecule. Hence, some peaks that could be co-eluted in scan
245 mode can be integrated with a value of resolution greater than 1. Results were expressed
246 as percentages of the total area represented by each one of the volatile compounds.
247

248 2.4. Physico-chemical and microbiological analyses of olive brines

249 The pH, free acidity, and combined acidity of samples were measured using a
250 Metrohm 670 Titroprocessor (Herisau, Switzerland). Free acidity was determined by
251 titrating up to pH 8.3 with 0.2N NaOH and expressed as percent (w/v) of lactic acid.
252 Combined acidity was determined with 2N HCl until the pH value reached 2.6 and
253 expressed as the equivalent of sodium hydroxide per liter. Sodium chloride by titration
254 with $AgNO_3$ and reducing sugars by the Fehling´s test were determined as described by
255 Fernández-Díez et al. (1985). Total polyphenols were measured with the Folin-
256 Ciocalteau reagent following the procedure described by Casado, Sánchez, Rejano, and
257 Montaño (2007).
258 The microbial population was determined by plating the brines on the
259 appropriate solid media, both by spreading 0.1 mL onto the surface and plating their
260 decimal dilutions (in 0.1% peptone water) with a Spiral Plater (Don Whitley Sci. Ltd.,
261 Shipley, England). De Man, Rogosa, Sharpe (MRS) agar with and without 0.02%
262 sodium azide was used for the lactic acid bacteria (LAB) determination, and
263 oxytetracycline-glucose-yeast extract (OGYE) agar was used for yeasts. Plates were
264 incubated at 32 ºC (MRS) or 26 ºC (OGYE) for up to 5 days, and the colony numbers
265 were recorded.
266

267 2.5. HPLC analyses

268 Organic acids (lactic, acetic and succinic acids) and ethanol were analyzed by
269 HPLC using a C18 column and deionized water (pH adjusted to 2.2 using concentrated
270 H_3PO_4) as the mobile phase (Sánchez, de Castro, Rejano, & Montaño, 2000).
271 Carbohydrates (sucrose, glucose, fructose, and mannitol) were determined by HPLC
272 using a Rezex RCM Monosaccharide column (Phenomenex, Torrance, CA) and
273 deionized water as the mobile phase (Casado and Montaño, 2008).
274

275 2.6. Statistical analyses

276 All the data were compiled and calculated using a combination of Microsoft
277 Excel 2010 (Microsoft Corporation, Redmond, WA) and Statistica software version 7.0
278 (Statsoft Inc., Tulsa, OK). The ANOVA test of Student-Newman-Keuls of multiple
279 comparisons of mean values was applied to the results to ascertain possible significant
280 differences among the samples studied. Significant differences were determined at the
281 $p<0.05$ level. In order to reveal any grouping of the table olives based on the
282 composition of volatile compounds, as well as to identify the main components
283 contained within each group, the data were subjected to principal component analysis
284 (PCA).

285 **3. Results and Discussion**

286 3.1. Selection of sample preparation methodology for HS-SPME analysis

287 As expected, the addition of salting-out agents such as NaCl improved extraction
288 efficiency (Fig. 1a). This can be attributed to a lower solubility of analytes in solution,
289 thus increasing the amount of sorbed analytes on the fiber (Balasubramanian and

290 Panigrahi, 2011). The preparation mode 3 did not have a significant effect on the
291 extraction efficiency of volatile compounds from olive pulp in comparison with
292 preparation mode 2, although a better precision was apparent. The number of detected
293 compounds for preparations 1 to 3 did not differ significantly (average ± SD was 156 ±
294 6, 154 ± 6, and 161 ± 5, respectively). Since enzymatic reactions and consequent
295 oxidation due to polyphenol-oxidases are supposedly absent in preparation mode 3 due
296 to the presence of ascorbic and citric acids (Aprea et al., 2012), the above result
297 suggests that these types of reactions (assuming these reactions occur during preparation
298 mode 2) do not contribute significantly to the volatile profile. In other words, this means
299 that the formation of volatile compounds did not occur during the homogenization step
300 of olive pulp, prior to SPME.

301 Sample dilution affected the extraction efficiency (Fig. 1b), which can be due to
302 improved agitation conditions at higher sample dilutions with a noticeable influence on
303 repeatability (RSD were 15.4, 18.7, and 4.3% for dilutions 1:1, 1:2, and 1:3,
304 respectively). It is well known that, in SPME, extraction increases with the stirring rate
305 of the aqueous phase (Zhang & Pawliszyn, 1993). Taking into account these results, a
306 simple sample dilution 1:3 with 30% NaCl was chosen as the optimum sample
307 preparation for the analysis of volatile compounds from Spanish-style green table olives
308 by SPME-GC-MS.

309

310 3.2. Chemical and microbiological characteristics of table olive samples

311

312 Physico-chemical and microbiological characteristics along with concentrations
313 of free sugars, organic acids, and ethanol in the different samples of Spanish-style green
314 olives after 5 months of brining are shown in Table 1. As expected, the main

315 fermentation substrates (glucose, fructose, sucrose) were totally metabolized. Mannitol,
316 another free carbohydrate present in raw olives (Montaño, Sánchez, López-López, de
317 Castro, & Rejano, 2010), was detected in small amounts (<0.1% in general). Final
318 values of physicochemical parameters and fermentation end-products in all samples
319 were within the normal ranges found in brines of Spanish-style green olives in bulk
320 (Montaño, Sánchez, Casado, de Castro, & Rejano, 2003). The only exception was the
321 sample HE, which contained a relatively high content of ethanol. This is consistent with
322 a greater growth of yeasts in detriment of the LAB, which could be partly inhibited by a
323 greater content of phenolic compounds (5.2 g/L expressed as gallic acid in HE versus
324 2.5-4.0 g/L in the remaining samples). In turn, this could be a consequence of applying
325 a less efficient washing step after lye treatment of olives.
326
327 3.3. Volatile compounds of table olive samples
328
329 A total of 102 individual aroma compounds were identified using the HS-SPME-
330 GC-MS technique (Table 2). The compounds were grouped into the following chemical
331 classes: alcohols, esters, acids, aldehydes, terpenes/terpenoids, phenols, hydrocarbons,
332 and other compounds. Most of the identified compounds (82 out of 102) had been
333 previously found as volatile compounds in one or various types of table olives,
334 including Spanish-style (Montaño et al., 1992; Sabatini and Marsilio, 2008; Iraqi et al.,
335 2005; Cano-Lamadrid et al., 2015), Greek-style (Sabatini, Mucciarella, & Marsilio,
336 2008; Randazzo et al., 2014; Bleve et al., 2014, 2015; De Angelis et al., 2015;
337 Martorana et al., 2015), Tunisian-style (Dabbou et al., 2012), Californian-style
338 (Sansone-Land, Takeoka, & Shoemaker, 2014), “alcaparras” stoned olives from
339 Portugal (Malheiro et al., 2011), “Greek-style” Moroccon black olives (Collin et al.,

2008) and “Campo Real” unfermented olives (Navarro et al., 2004). Twenty compounds (isopropyl alcohol, 3-methyl-1-pentanol, 1-octen-3-ol, methyl butanoate, methyl 3-methylbutanoate, methyl (E)-3-hexenoate, methyl lactate, isoamyl lactate, ethyl salicylate, ethyl 3-cyclohexenecarboxylate, (E)-3-hexenoic acid, decanoic acid, benzoic acid, dihydroedulan, isogeraniol, geraniol, iridomyrmecin, *p*-propyl guaiacol, eugenol, and tyrosol) were reported for the first time as volatile compounds in table olives. Although it is known that the last compound (tyrosol) is normally present in table olives as a result of the hydrolysis of ligstroside (a heterosidic ester of tyrosol and elenolic acid) (Brenes, Rejano, García, Sánchez, & Garrido, 1995), to the best of our knowledge, its detection as a headspace volatile component has not been previously reported.

The total amounts of the identified chemical classes in the different samples are shown in Fig. 2. The headspace profile was predominated by alcohols and phenols, followed by acids and esters, whereas the relative amounts of the remaining classes were quite lower (< 5% in general). Cano-Lamadrid et al. (2015), using an SPME method similar to ours, reported that aldehydes were one of the most abundant families in the volatile profiles of Spanish-style green table olives from Manzanilla cultivar. This discrepancy with our study may be due to the different origins of olives and differences in microbial growth during fermentation. Climatic and agronomic conditions of olive growing can affect volatile composition in case of virgin olive oils obtained by the same cultivar (Angerosa et al., 2004). Formation of volatile compounds in Spanish-style (or Greek-style) table olives is a dynamic process that develops mainly by indigenous lactic acid bacteria and yeasts, together with a variety of contaminating microorganisms (Sabatini & Marsilio, 2008). However, the fermentation process is not fully predictable. It has been reported that differences in the fermentation process affect the concentrations of volatile compounds in Greek-style green table olives (De Angelis et

al., 2015). The ANOVA study showed that the effect of cultivar was significant ($p<0.05$) for all chemical classes, with the exception of alcohols (data not shown). In samples from the Manzanilla cultivar, there were significant differences between the samples for all chemical classes, with the exception of esters and terpenes/terpenoids (). In case of Gordal cultivar, significant differences between the samples were only found for terpenes and "other compounds" However, we must mention that HS-SPME analyses of duplicate fermenters of sample GU were coincident with the SPME fiber death, which forced us to change the fiber. This could explain the high standard errors for different chemical classes in this sample (Fig. 2). It is known that the change of fiber in a study can negatively affect the reproducibility especially for fibers from different batches (Kalua, Bedgood, & Prenzler, 2006).The most pronounced effect occurred in the Hojiblanca cultivar, where significant differences between the samples occurred for all chemical classes. Sample HE was characterized by a higher content of alcohols compared to samples HA and HC, which agrees with the higher content of ethanol in HE mentioned in the previous section. In addition, clear differences in other chemical classes were found in HE in comparison with HA and HC. It appears that differences in the fermentation process significantly affect the volatile profile of product.

Regarding individual volatile compounds, the relative amounts of 102 volatile compounds, expressed as percentage of the total peak area, for the different samples are shown in Table 3. Compounds are ordered according to their chemical class.

3.3.1. Alcohols

Alcohols are compounds formed from enzymatic reactions during fruit ripening and from heterolactic and alcoholic fermentation during olive processing. In our study,

20 alcohols were identified, with phenylethyl alcohol (representing 8-19% of all volatile compounds in the headspace), benzyl alcohol (3.1-8%), (Z)-3-hexen-1-ol (2.7-5.8%), and ethanol (1.2-13.1%) being the major ones in all the samples. Phenylethyl alcohol is an aromatic alcohol with a rose-like odor and occurs in many essential oils and fermented foods. It is likely that this alcohol in Spanish-style green olives is formed, at least in part, as a result of yeast fermentation, as yeast species such as *Saccharomyces cerevisiae* could produce phenylethyl alcohol from L-phenylalanine (Eshkol, Sendovski, Bahalul, Katz-Ezov, Kashi, & Fishman, 2009). Benzyl alcohol is naturally synthesized by many plants, notably accumulating in edible fruits and tea leaves (CoE, 1992). The presence of 1-hexanol and (Z)-3-hexen-1-ol, which are higher alcohols from the lipoxygenase pathway (Siegmund, 2015), may be due to a lipoxygenase-like metabolism of polyunsaturated fatty acids, affected by enzymes produced in the brine medium by lactic acid bacteria and yeasts together with other different microorganisms (Sabatini & Marsilio, 2008). Ethanol can be classified as a fermentation-derived compound, which is produced in table olives via yeasts and hetero-fermentative lactic acid bacteria from sugars (Sabatini and Marsilio, 2008). The relatively high content of ethanol in sample HE agrees with results obtained by HPLC and microbiological analysis, which indicates that fermentation process is mostly produced by yeasts. As a consequence, the number of alcohols showing significant differences between the samples was higher in the Hojiblanca cultivar (a significant effect was found for 19 out of 20 alcohols) compared to Manzanilla (12 out of 20) and Gordal (6 out of 20) cultivars (Table 3).

3.3.2. Esters

The largest group of volatile compounds identified in our study was esters of which there were 29 compounds. Volatile esters are major components of the aroma of all fruits, and are sometimes mainly responsible for the pleasant flavor appreciated by consumers (Sabatini and Marsilio, 2008). Their formation and content mainly depend on the number of alcohols and acids. Acetate esters and propanoate esters could be synthesized by the esterification of volatile alcohols with acetyl-CoA and propionyl-CoA, respectively (Sabatini and Marsilio, 2008). Ethyl and methyl esters were the most numerous esters, with ethyl acetate being the dominant compound in all samples (representing 0.8-8% of all volatile compounds). Ethyl lactate was relatively important in samples from the Gordal cultivar and sample HE (representing more than 1% of all volatile compounds). In the latter sample, this could be explained by its high content of ethanol, as shown in Table 1. The presence in sample HE of relatively high amounts of ethyl octanoate (6%), ethyl hexanoate (2%), ethyl decanoate (1.2%), and methyl octanoate (1%) is noteworthy. As occurred with alcohols, the number of alcohols showing significant differences between the samples was higher in olives from Hojiblanca and Manzanilla cultivars compared to Gordal cultivar (Table 3).

3.3.3. Volatile acids

Within the family of volatile acids, 11 compounds were identified. Acetic acid was the dominant acid in all cases, representing 8-14% of all volatile compounds in the headspace. It is known that this acid is formed in olives during the lye treatment step, presumably from fragmentation by alkali from other compounds, and during the fermentation step (Sánchez et al., 2000). The content of propanoic acid was relatively high in samples MC, MAm, GU, HA, and HC (6-12%); in the remaining samples its

content was low (0-1%). The formation and content of this acid depends on the growth of *Propionibacterium* species, characteristic of the "fourth stage" of fermentation in Spanish-style table olives (Montaño et al., 2003). Among the remaining acids identified, it is worth mentioning that 2-methylbutanoic acid was present at a 0.6-2.5% level. For each cultivar, its content was significantly different between the samples studied (Table 3). On the contrary, the content of benzoic acid was not significantly different between the samples in any cultivar. However, since no significant differences in benzoic acid were found among cultivars according to ANOVA (data not shown), this acid is not considered a good candidate for marker of olive cultivar in Spanish-style green olives. Hexanoic, octanoic and decanoic acids were present at relatively high amounts in HE compared to the other samples, which is consistent with the high contents of the corresponding ethyl esters, as mentioned above.

3.3.4. Aldehydes

Among 8 aldehydes identified, benzaldehyde was the most abundant, representing 0.5-1.2% of all volatile compounds. This aldehyde may result from enzymatic reactions during fruit ripening, and is present in intact raw olives (Malheiro, Casal, Cunha, Baptista, & Pereira, 2015), but benzaldehyde formation during the fermentation phase of Spanish-style olives should not be ruled out. *Lactobacillus plantarum*, the main species of LAB during fermentation, has been reported to convert phenylalanine to benzaldehyde (Nierop Groot and De Bont, 1998). For each olive cultivar, the benzaldehyde content in Spanish-style olives was significantly different between the samples studied (Table 3). The contrary occurred in the case of octanal and (E)-2-decenal. In addition, the mean content of the latter compound was significantly

different among cultivars (Manzanilla > Gordal > Hojiblanca). It suggests that (E)-2-decenal could be used as a potential marker of olive cultivar in Spanish-style green olives. However, a greater amount of data is necessary to corroborate this hypothesis.

3.3.5. Terpene/terpenoids

Terpene compounds consisted of oxygenated as well as non-oxygenated monoterpenes, sesquiterpenes and irregular terpenes, which all occurred in relatively low amounts in the headspace of the samples. All of these compounds can be classified as olive-derived compounds. The oxygenated monoterpenes detected included the alcohols linalool, linalool oxide, α-terpineol, geraniol, and isogeraniol; and the iridoid monoterpene iridomyrmecin. The non-oxygenated terpenes consisted of the common monoterpene limonene and the sesquiterpenes copaene, caryophyllene, cycloisosativene, and α-muurolene. The irregular terpenes detected included 6-methyl-5-hepten-2-one, dihydroedulan, and β-damascenone, which are most likely formed from carotenoids. Of the 14 terpene compounds identified, dihydroedulan, geraniol, isogeraniol, and iridomyrmecin were identified for the first time in table olives. As found for other chemical classes, the contents of most terpene compounds were not significantly different between the samples from Gordal cultivar (Table 3). However, Manzanilla and Hojiblanca cultivars showed significant changes in most of the terpene compounds. In particular, copaene and α-muurolene showed significant changes between the samplesfor all three cultivars. Although the size of our data set is too small and the stability of these compounds during table olive processing has not been evaluated, it suggests that these sesquiterpenes could be considered as potential molecular marker candidates or play a role in determining the authenticity and

protection of regional produce. In fact, copaene and α-muurolene, along with α-farnesene, have been proposed as markers of extra virgin olive oil origin (Damascelli and Palmisano, 2013).

3.3.6. Volatile phenols

Within the volatile phenols, 9 compounds were identified including 5 guaiacol derivatives (p-creosol, p-ethyl guaiacol, p-propyl guaiacol, eugenol, vanillin) and 4 phenol derivatives (phenol, p-cresol, 4-ethyl phenol, tyrosol). The most abundant compound in all the samples was p-creosol (26-37%, except in sample HE). The content of 4-ethyl phenol was also relatively important in all samples (1-5%). Most of these compounds are likely formed during the fermentation process as a result of the activity of microorganisms. Thus, the presence of volatile phenols in olive oils with strong fusty, musty, and muddy defects as well as in stored olive paste has been attributed to microbial activity (Vichi, Romero, Gallardo-Chacón, Tous, López-Tamames, & Buxaderas, 2009). It is known that certain strains of LAB, *L. plantarum* among them, are able to produce volatile phenols from the metabolism of phenolic acids (Silva, Campos, Hogg, & Couto, 2011). Changes in individual phenols were particularly important in the case of the Manzanilla cultivar (significant differences between the samples were found for 7 out of 9 phenols, Table 3). For each cultivar, the contents of tyrosol were not significantly different between the samples studied.

3.3.7. Hydrocarbons

514 Five hydrocarbons (octane, decane, o-xylene, styrene, and 2-bornene), all of
515 them previously detected in table olives, were identified in all samples. In general, 2-
516 bornene, a bridge cyclic hydrocarbon previously detected in Spanish-style olives (Iraqi
517 et al., 2005) was the most abundant (0.5-1.6%), with contents significantly different
518 between the samples from Manzanillaor Hojiblanca cultivar (Table 3).
519
520 3.3.8. Other volatile compounds
521
522 Finally, other volatile compounds identified in our study were dimethyl sulfide,
523 dimethyl sulfoxide, butyrolactone, 1,4-dimethoxybenzene, and the stereoisomeric
524 compounds theaspirane A and B. In general, the major compounds were dimethyl
525 sulfide and theaspirane. On the other hand, 1,4-dimethoxybenzene was only found in
526 samples from the Hojiblanca cultivar and at low concentrations (0.04-0.05%), but
527 further research is needed to know if this compound could be considered as a potential
528 marker candidate of Spanish-style green olives elaborated with the Hojiblanca cultivar.
529 Dimethyl sulfide contents were significantly different between the samples from
530 Manzanilla or Hojiblanca cultivar. Mean contents of theaspirane A and B were
531 significantly different among cultivars (Hojiblanca > Manzanilla > Gordal) while the
532 differences between the samples studied for each cultivar were small or not significant.
533
534 3.3.9. Principal component analysis (PCA) of volatile compounds
535
536 PCA was performed using the contents of individual volatile compounds as the
537 variables. For this study, the sample HE was not considered due to its distinct
538 processing operations and final characteristics in comparison with the other samples.

539 The first two principal components accounted for 52.36% of the variation in the data.
540 The score plot showed that three separate groups were clearly visible (Fig 3a): all
541 samples from the Gordal cultivar (GU1, GU2, GA1, GA2) formed one group; all
542 samples from the Manzanilla cultivar (MC1, MC2, MAl1, MAl2, MAm1, MAm2)
543 formed a second group, and the third group was composed of samples from the
544 Hojiblanca cultivar (HA1, HA2, HC). The locations where the fruits were grown for a
545 given cultivar were not clearly distinguished, indicating that the fruit growing
546 environment had a minor influence on the volatile composition of Spanish-style green
547 table olives. Similarly, in virgin olive oil, it has been reported that cultivar is the
548 dominant factor in the formation of the aroma whereas the fruit grown environment has
549 little effect (Angerosa et al., 2004). The loading plot (Fig. 3b) showed that the volatile
550 compounds mainly associated with the first group were the esters ethyl acetate (21),
551 ethyl lactate (38) and ethyl benzoate (44). The second group was particularly related
552 to1-octanol (17), phenylethyl alcohol (20) and (E)-2-decenal (68), while the third group
553 was mainly related to propyl acetate (23) and 1,4-dimethoxybenzene (102).
554

555 **4. Conclusions**

556 In this study, the volatile profiles of Spanish-style green olives prepared from
557 Manzanilla, Gordal and Hojiblanca cultivars each grown at different locations in Spain
558 were evaluated using HS-SPME-GC-MS. All samples presented complex aroma
559 profiles rich in different families of aroma compounds, mainly alcohols and phenols.
560 More than 100 volatile compounds distributed over different chemical groups were
561 identified in the pulp of olives. The major volatile compounds characterizing the
562 headspace for most samples were: p-creosol, phenylethyl alcohol, acetic acid, ethanol,
563 benzyl alcohol, ethyl acetate, and (Z)-3-hexen-1-ol. Based on the content of individual

564 volatile compounds and PCA, the samples were clearly separated according to their
565 olive cultivar. However, the different locations of samples for each cultivar were poorly
566 distinguished. The contents of benzoic acid, octanal, (E)-2-decenal, and tyrosol were
567 not significantly different between the samples studied for each cultivar, but only (E)-2-
568 decenal showed significant differences among the three cultivars. Therefore, this
569 aldehyde would be a promising candidate as marker of olive cultivar in Spanish-style
570 green table olives. However, further studies are needed to support the results obtained
571 by this first screening. Apart from this, new experiments are in progress in our
572 laboratories to determine the contribution of each volatile compound to the
573 characteristic aroma of Spanish-style green table olive and to elucidate the relationship
574 between aroma compounds and sensory attributes.

575

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579

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732 FIGURE CAPTIONS

733

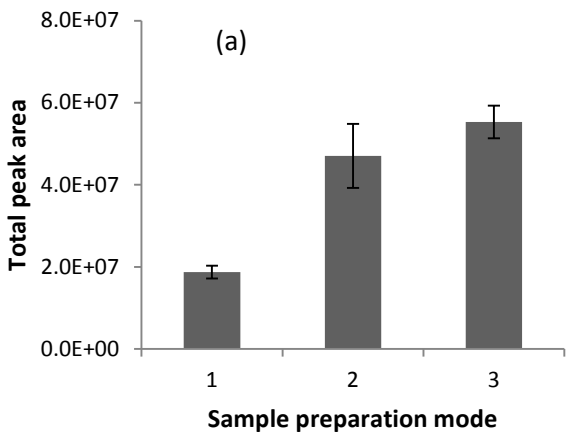
734 Figure 1. Influence of (a) sample preparation mode and (b) sample dilution on HS-
735 SPME extraction efficiency of volatile compounds in pulp of Spanish-style green table
736 olives (Manzanilla cultivar) under constant stirring (600 rpm). Experiments are
737 described in text. Error bars indicate 95% confidence intervals (n = 3). Sample
738 preparation modes: (1) 5 g of pulp + 5 mL of water, (2) 5 g of pulp + 5 mL of 30%
739 NaCl, and (3) 10 g aliquot of a mix composed of 20 g of pulp + 20 mL of a solution
740 containing 30% (w/v) NaCl, 0.3% (w/v) ascorbic acid and 0.3% (w/v) citric acid.

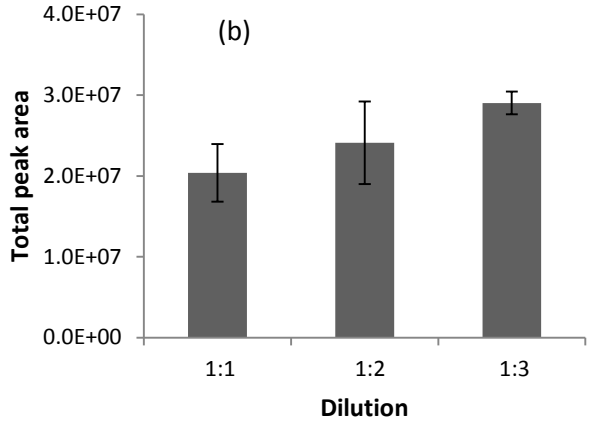
741

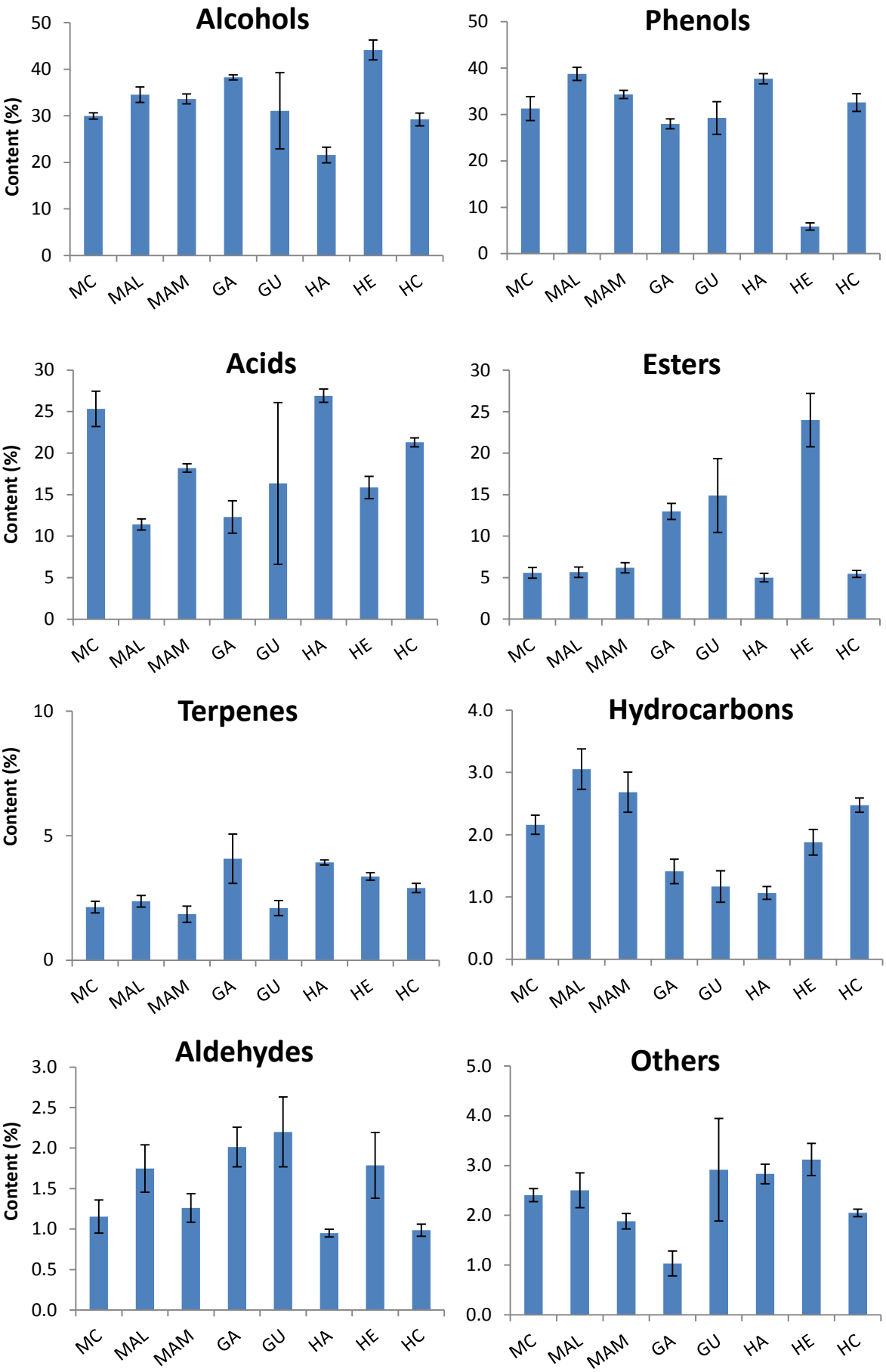
742 Figure 2. Chemical classes of the volatile compounds in Spanish-style green olives
743 obtained with olives from cultivars Manzanilla, Gordal, and Hojiblanca grown at
744 different locations. Sample codes are described in text. Error bars indicate 95%
745 confidence intervals (n = 6).

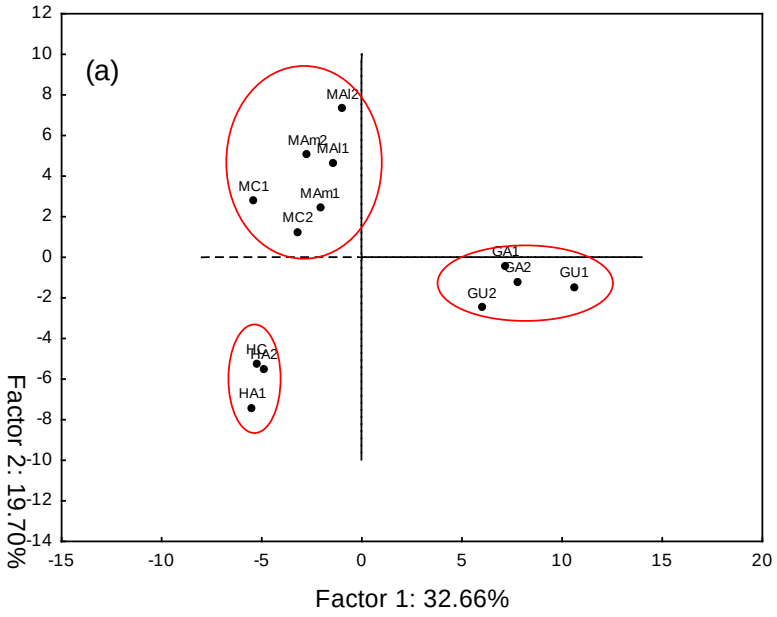
746

747 Figure 3. Principal component analysis (PCA) performed on individual volatile
748 compounds: (a) distinction between the samples (scores); (b) relationships between the
749 variables (loadings).


(a)
8.0E+07
6.0E+07
4.0E+07
2.0E+07
0.0E+00
Total peak area
1
2
3
Sample preparation mode


(b)
4.0E+07
3.0E+07
2.0E+07
1.0E+07
0.0E+00
Total peak area
1:1
1:2
1:3
Dilution


Alcohols
Phenols
Acids
Esters
Terpenes
Hydrocarbons
Aldehydes
Others
Content (%)
MC
MAL
MAM
GA
GU
HA
HE
HC


(a)
MAl2
MAm2
MAl1
MC1
MAm1
MC2
GA1
GA2
GU1
GU2
HC
HA2
HA1
Factor 2: 19.70%
Factor 1: 32.66%

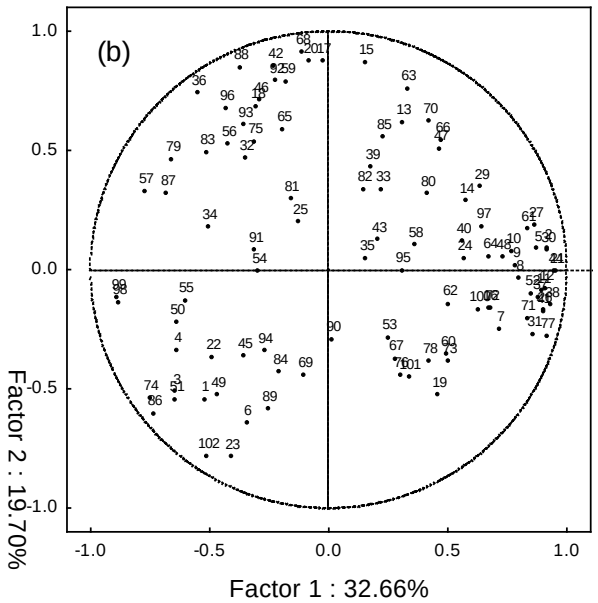

(b)
Factor 2 : 19.70%
Factor 1 : 32.66%

Table 1. Physico-chemical and microbiological characteristics, and substrates and end-products of fermentation after 5 months of brining

	Samples[a]							
	MC	MAl	MAm	GA	GU	HA	HE	HC
Physico-chemical parameter								
pH	4.03± 0.01c	3.80± 0.04b	4.01 ±0.03c	3.69 ±0.02a	3.68 ±0.04a	3.88 ±0.01b	3.85 ±0.04b	3.91 ±0.00b
Titratable acidity (%)	0.82 ±0.02a	0.9 ±0.1ab	0.71 ±0.04a	1.05 ±0.01ab	0.98 ±0.00ab	1.2 ±0.1bc	1.3 ±0.1c	1.0 ±0.0ab
Combined acidity (N)	0.11 ±0.00c	0.09 ±0.00a	0.10 ±0.00b	0.09 ±0.00a	0.09 ±0.00a	0.13 ±0.00d	0.14 ±0.00e	0.11 ±0.00c
Salt (% NaCl)	5.6 ±0.2a	5.95 ±0.01b	6.0 ±0.1b	5.8 ±0.1ab	6.3 ±0.1c	5.59± 0.01a	5.7 ±0.1ab	5.70 ±0.00ab
Total phenols (g/L gallic acid)	3.6±0.3c	4.0±0.3d	3.4±0.5c	2.9±0.3b	2.5±0.2a	2.9±0.5b	5.2±0.4e	3.4 ±0.2c
Microbial population (log cfu/mL)								
Lactic acid bacteria	6.8±0.1d	6.4±0.1c	6.6±0.2cd	6.4±0.0c	5.7±0.2b	5.7±0.2b	<3.3a	5.9 ± 0.0b
Yeasts	2.2±0.3a	2.7±0.1a	4.0±0.1b	4.1±0.3b	3.7±0.8ab	4.8±0.8bc	5.6±0.0c	3.4 ± 0.2b
Substrates and end-products of fermentation (g/L)[b]								
Mannitol	0.05 ±0.00a	0.09 ±0.01a	0.05 ±0.01a	1 ± 1a	0.7 ±0.5a	0.05 ±0.00a	0.6 ±0.3a	0.07 ±0.01a
Lactic acid	13.66± 0.08b	14.3±0.9b	11.4± 0.5a	18.6± 0.1d	16.8± 0.3c	17.2± 0.6c	19.9± 0.3e	15.1 ±0.1b
Acetic acid	1.92 ±0.05ab	0.96±0.01b	1.44 ±0.03ab	1.0±0.2a	0.9±0.2a	2.2± 0.2b	1.7 ±0.1ab	2.4 ±0.1b
Succinic acid	0.50± 0.02bc	0.11±0.01a	0.30± 0.01ab	0.29 ±0.02ab	0.3±0.1a	0.29 ± 0.00ab	0.6± 0.2c	0.34 ±0.02ab
Ethanol	0.61 ± 0.06 a	0.3 ± 0.1a	1.1 ± 0.1a	1.3 ± 0.2a	1.1 ± 0.2a	0.21 ± 0.02a	3.2 ± 0.9b	0.25 ±0.01a

[a]Values are means ± SD of two fermenters, each analysed in duplicate, except for sample HC whose values are from one fermenter. [b] Glucose, fructose and sucrose were not detected in any case. Data in the same row with different letters are significantly different ($p < 0.05$).

Table 2. Volatile compounds in the headspace of Spanish-style green table olives, processed with olives from different cultivars grown at different locations, identified in the present study.

Code	Compound	LRI [a]	IEC (*m/z*) [b]	ID [c]	Ref. [d]
Alcohols					
1	Isopropyl alcohol	928	45	A	-
2	Ethanol	935	45	A	1,3,4,5,10,13,15,16,17
3	2-Butanol	1025	59	A	3,4,5,7,10,13,15,17
4	1-Propanol	1039	59	A	3,4,5,7,8,13
5	Isobutanol	1107	43	A	3,7,8,13,17
6	1-Butanol	1153	56	A	3,7,13,17
7	Isopentanol	1211	55	A	2,3,6,7,8,9,11,13,14,16,17
8	3-Methyl-3-buten-1-ol	1256	68	A	7,8,17
9	1-Pentanol	1255	55	A	2,3,7,8,9,12,13
10	3-Methyl-2-buten-1-ol	1324	71	A	7,8,12,17
11	3-Methyl-1-pentanol	1329	56	A	-
12	1-Hexanol	1356	56	A	1,2,3,6,7,8,9,10,11,13,14,16,17
13	(Z)-3-Hexen-1-ol	1385	67	A	1,2,3,6,7,8,9,11,12,13,14,15,17
14	1-Octen-3-ol	1454	57	A	-
15	1-Heptanol	1458	70	A	2,7,8,9,10,12
16	2-Ethyl-1-hexanol	1491	57	A	9,16,17
17	1-Octanol	1560	84	A	1,6,9,12,17
18	1-Nonanol	1661	70	A	2,8,9,12
19	Benzyl alcohol	1871	108	A	2,6,7,8,9,12,14,17
20	Phenylethyl alcohol	1903	91	A	1,2,6,9,12,14,16,17
Esters					
21	Ethyl acetate	897	43	A	3,4,5,7,8,12,13,16,17
22	Methyl propanoate	911	57	A	16,17

23	Propyl acetate	976	61	A	1,3,7,8,9,13,17
24	Methyl butanoate	989	87	A	-
25	Methyl 2-methylbutanoate	1010	88	A	17
26	Isobutyl acetate	1013	43	A	8,17
27	Methyl 3-methylbutanoate	1018	74	A	-
28	Ethyl butanoate	1033	71	A	7,8,16,17
29	Ethyl 2-methylbutanoate	1048	102	A	7,8,9,11,14,17
30	Ethyl 3-methylbutanoate	1064	88	A	7,8,9,14,17
31	Isoamyl acetate	1118	43	A	7,8,9,11,14,17
32	Methyl hexanoate	1185	74	A	7,11,12,17
33	Ethyl hexanoate	1231	88	A	7,8,9,15,16,17
34	Methyl (E)-3-hexenoate	1259	128	A	-
35	Hexyl acetate	1268	56	A	1,2,7,8,9,10,11,12,17
36	Ethyl (E)-3-hexenoate	1301	142	A	8
37	Methyl lactate	1322	45	A	-
38	Ethyl lactate	1345	75	A	6,7,16
39	Methyl octanoate	1387	74	A	7,9,12
40	Ethyl octanoate	1432	88	A	1,7,8,9,16,17
41	Isoamyl lactate	1566	45	A	-
42	Methyl decanoate	1592	87	A	9
43	Ethyl decanoate	1635	88	A	9
44	Ethyl benzoate	1654	105	A	9
45	Benzyl acetate	1721	108	A	6,8,9
46	Methyl salicylate	1758	120	A	6,8,9,10,17
47	Ethyl salicylate	1792	120	A	-
48	Methyl hydrocinnamate	1834	104	A	6
49	Ethyl 3-cyclohexenecarboxylate	2182	81	C	-

Acids					
50	Acetic acid	1460	60	A	1,3,6,7,8,12,13,16,17
51	Propanoic acid	1549	74	A	1,3,6,7,8,13,16,17
52	Isobutanoic acid	1581	43	A	2,7,8,14,16,17
53	Butanoic acid	1640	73	A	6,16,17
54	2-Methylbutanoic acid	1680	74	A	1,7,8,14,17
55	Hexanoic acid	1854	73	A	6,17
56	Heptanoic acid	1959	73	A	6,17
57	(E)-3-Hexenoic acid	1966	114	A	-
58	Octanoic acid	2065	73	A	6,10,12,17
59	Decanoic acid	2277	73	A	-
60	Benzoic acid	2436	105	A	-
Aldehydes					
61	Pentanal	980	44	A	9,11
62	Heptanal	1177	70	A	8,9,10,11
63	Octanal	1284	84	A	1,2,6,9,11,12,16,17
64	2-Heptenal	1313	83	A	1,2,9,11,14
65	Nonanal	1388	98	A	1,2,3,6,7,8,9,11,13,14,16,17
66	(E)-2-Octenal	1420	55	A	9,11,12
67	Benzaldehyde	1511	106	A	1,2,6,7,8,9,10,11,14,15,16,17
68	(E)- 2-Decenal	1633	70	A	1,6,8,9,10,11,12,14
Terpenes and terpenoids					
69	Limonene	1182	93	A	1,2,9,10,11,14,17
70	6-Methyl-5-hepten-2-one	1334	108	A	1,2,7,9,10,11,17
71	Linalool oxide	1434	94	A	8,17

72	(+)-Cycloisosativene	1459	161	B	9,
73	Copaene	1477	119	B	2,9,10,11,12
74	Dihydroedulan	1501	179	B	-
75	Linalool	1551	93	A	1,2,8,9,10,15
76	Caryophyllene	1575	133	A	2,10,11
77	α-Terpineol	1690	93	A	2,6,8,9,14
78	α-Muurolene	1707	161	C	9,11,12
79	β-Damascenone	1805	121	A	9,10,14
80	Isogeraniol	1811	121	B	-
81	Geraniol	1847	69	A	-
82	Iridomyrmecin	2129	95	B	-
Phenols					
83	p-Creosol	1949	138	A	9,16,17
84	Phenol	2006	94	A	6,9,17
85	p-Ethyl guaiacol	2022	137	A	9,14,16,17
86	p-Cresol	2083	107	A	9,16
87	p-Propyl guaiacol	2100	137	A	-
88	Eugenol	2158	164	A	-
89	4-Ethyl phenol	2175	107	A	1,2,6,9,16,17
90	Vanillin	2541	151	A	6,12,14
91	Tyrosol	2804	107	A	-
Hydrocarbon*s*					
92	Octane	807	85	A	1,2,8,9,10,17
93	Decane	1001	57	A	2
94	o-Xylene	1170	91	A	12
95	Styrene	1249	104	A	2,6,7,8,9,14

96	2-Bornene	1505	121	C	2
Other compounds					
97	Dimethyl sulfide	765	62	A	1,5,7
98	Theaspirane A	1484	138	A	9
99	Theaspirane B	1524	138	A	9
100	Dimethyl sulfoxide	1556	63	A	2
101	Butyrolactone	1613	42	A	6,7,8
102	1,4-Dimethoxybenzene	1730	123	A	1

[a] Linear retention index on VF-Wax column.
[b] Ion extraction chromatogram, *m/z* used to obtain the GC peak area of each compound.
[c] Identification: A, identified, mass spectrum and RI were in accordance with standards; B, tentatively identified, mass spectrum matched in the standard NIST 2008 library and RI matched with the NIST Standard Reference Database (NIST Chemistry WebBook); C, tentatively identified, mass spectrum agreed with the standard NIST 2008.
[d] Previously reported as volatile compound in typical Spanish-style green table olives (1, Cano-Lamadrid et al., 2015; 2, Iraqi et al., 2005; 3, Sabatini and Marsilio, 2008; 4, Montaño et al., 1992; 5, Vergara et al., 2013) or other preparations of table olives (6, Martorana et al., 2015; 7, Bleve et al., 2015; 8, Bleve et al., 2014; 9,Sansone-Land et al., 2014; 10, Dabbou et al., 2012; 11, Malheiro et al., 2011; 12, Aponte et al., 2010; 13, Sabatini et al, 2008; 14, Collin et al., 2008; 15, Navarro et al., 2004; 16, Randazzo et al., 2014; 17, De Angelis et al., 2015). -, not reported.

Table 3. Volatile compounds in the headspace of Spanish-style green table olives from Manzanilla, Gordal and Hojiblanca cultivars as affected by olive growing location

		Content (% of total area of identified compounds)[a]									
		Manzanilla cultivar				Gordal cultivar			Hojiblanca cultivar			
		MC	MAL	MAM	P[b]	GA	GU	P[b]	HA	HE	HC	P[b]
	Alcohols											
1	Isopropyl alcohol	0.6 b	nd a	0.03 a	***	nd	nd		0.8 b	nd a	2.7 c	***
2	Ethanol	2.7 a	3.4 b	3.8 b	**	8.5	6.7	ns	1.2 a	13.1 b	1.4 a	***
3	2-Butanol	0.5 b	0.07 a	0.3 a	***	0.01 a	0.2 b	*	0.6 b	0.02 a	1.4 c	***
4	1-Propanol	2.3 b	0.06 a	2.1 b	***	0.3	1.3	ns	2.5 b	nd a	1.4 b	***
5	Isobutanol	nd a	0.03 b	0.04 b	***	0.12	0.05	ns	nd a	0.3 b	nd a	***
6	1-Butanol	nd	0.01	tr	ns	0.02	0.02	ns	0.2 b	0.04 a	0.07 a	*
7	Isopentanol	0.4 a	0.8 b	0.7 ab	***	2.4	2.1	ns	0.33 a	4.0 b	0.33 a	***
8	3-Methyl-3-buten-1-ol	0.09 b	0.07 ab	0.05 a	**	0.09	0.12	ns	0.06	0.05	0.03	ns
9	1-Pentanol	nd a	0.08 b	0.03 ab	***	0.08	0.10	ns	0.04 b	0.06 b	nd a	***
10	3-Methyl-2-buten-1-ol	0.14	0.12	0.11	ns	0.16	0.19	ns	0.11 b	0.18 c	0.03 a	**

11	3-Methyl-1-pentanol	nd a	0.04 b	nd a	***	0.08 a	0.14 b	***	tr a	0.02 b	nd a	***
12	1-Hexanol	0.5	0.6	0.7	ns	1.2	1.3	ns	0.4 a	0.6 b	0.8 c	***
13	(Z)-3-Hexen-1-ol	4.2	5.8	5.1	ns	4.0	4	ns	2.7 a	3.9 b	3.1 ab	**
14	1-Octen-3-ol	0.16	0.17	0.14	ns	0.17 a	0.3 b	*	0.06 a	0.15 b	0.12 b	**
15	1-Heptanol	0.13 a	0.25 b	0.28 b	***	0.15	0.06	ns	nd a	0.14 b	nd a	***
16	2-Ethyl-1-hexanol	0.09	0.09	0.09	ns	0.11 a	1.0 b	***	0.09 b	0.07 a	0.07 a	*
17	1-Octanol	0.22 a	0.36 b	0.37 b	***	0.21	0.22	ns	0.10 a	0.20 b	0.19 b	***
18	1-Nonanol	0.35 a	0.34 a	0.7 b	***	0.15 a	0.18 b	*	0.12 b	0.10 a	0.18 c	***
19	Benzyl alcohol	3.2	3.5	3.1	ns	5.9	5	ns	4.2 a	8 c	7.3 b	***
20	Phenylethyl alcohol	14	19	15.9	ns	15 b	8.7 a	*	8 a	12.7 c	10.6 b	***
	Esters											
21	Ethyl acetate	2.4	2.3	3.1	ns	6	7	ns	1.3 a	8 b	0.8 a	***
22	Methyl propanoate	0.6 b	0.03 a	0.7 b	***	0.08 a	0.6 b	*	0.8 c	0.01 a	0.39 b	***
23	Propyl acetate	0.26 c	nd a	0.18 b	***	0.1 a	0.7 b	*	1.4 b	0.01 a	1.8 c	***
24	Methyl butanoate	0.02	0.02	0.02	ns	0.03	0.04	ns	0.01 b	0.01 b	tr a	***
25	Methyl 2-methylbutanoate	0.13 b	0.08 a	0.05 a	***	0.06	0.07	ns	0.07 b	0.07 b	0.02 a	**

26	Isobutyl acetate	nd	nd	nd		0.06	0.03	ns	nd a	0.11 b	nd a	***
27	Methyl 3-methylbutanoate	0.04	0.07	0.06	ns	0.08 a	0.14 b	***	0.02 b	0.03 b	nd a	***
28	Ethyl butanoate	nd	nd	nd		nd	nd		nd a	0.10 b	nd a	***
29	Ethyl 2-methylbutanoate	0.12	0.12	0.10	ns	0.10 a	0.17 b	**	0.06 a	0.6 b	0.04 a	***
30	Ethyl 3-methylbutanoate	0.04 a	0.10 b	0.11 b	***	0.29	0.26	ns	0.01 a	0.19 b	0.02 a	***
31	Isoamyl acetate	0.09 a	0.13 b	0.22 c	***	0.6	0.51	ns	0.18 a	0.7 b	0.25 a	***
32	Methyl hexanoate	0.14	0.12	0.11	ns	nd a	0.3 b	**	nd a	nd a	0.18 b	***
33	Ethyl hexanoate	0.06	0.07	0.10	ns	0.10	0.07	ns	0.03a	2 b	0.09 a	***
34	Methyl (E)-3-hexenoate	0.03 b	0.02 ab	0.01 a	**	nd a	0.02 b	***	0.01 a	0.02 b	0.02 b	**
35	Hexyl acetate	0.01 a	0.04 c	0.03 b	***	0.03	0.03	ns	0.03a	0.02a	0.05 b	***
36	Ethyl (E)-3-hexenoate	tr	0.02 b	0.01 a	***	tr	tr		tr a	0.05 b	tr a	***
37	Methyl lactate	0.5 b	0.8 c	0.3 a	***	1.3	1.7	ns	0.5	0.5	0.4	ns
38	Ethyl lactate	0.2 a	0.4 b	0.19 a	***	1.9	2	ns	0.19 a	1.3 b	0.18 a	***
39	Methyl octanoate	0.08	0.06	0.07	ns	0.1	0.02	ns	0.04 a	1.0 b	0.01 a	***
40	Ethyl octanoate	0.05 a	0.07 ab	0.10 b	*	0.3	0.11	ns	0.01 a	6 b	0.05 a	**

41	Isoamyl lactate	nd	nd	nd		0.11	0.1	ns	nd a	0.15 b	nd a	***
42	Methyl decanoate	0.02	0.02	0.02	ns	0.01	nd	ns	nd a	0.2 b	nd a	***
43	Ethyl decanoate	0.02 a	0.03 a	0.04 b	**	0.1	nd	ns	nd a	1.2 b	0.05 a	***
44	Ethyl benzoate	0.05 a	0.08 b	0.11 c	***	0.28	0.2	ns	0.02 a	0.19 c	0.09 b	***
45	Benzyl acetate	0.15 a	0.23 b	0.16 a	***	0.19 b	0.15 a	**	0.24 a	0.3 a	0.53 b	***
46	Methyl salicylate	0.11 a	0.5 b	0.23 a	***	0.06 b	0.02 a	**	0.03 a	0.07 b	0.22 c	***
47	Ethyl salicylate	0.04 a	0.12 c	0.09 b	***	0.2 b	0.05 a	*	nd a	0.11 c	0.04 b	***
48	Methyl hydrocinnamate	0.5 b	0.24 a	0.26 a	***	0.4 a	0.70 b	**	0.14	0.2	0.2	ns
49	Ethyl 3-cyclohexenecarboxylate	0.03 b	0.01 a	0.01 a	***	0.01	0.02	ns	0.02 a	0.02 a	0.03 b	***
	Acids											
50	Acetic acid	14 b	8.8 a	8.7 a	***	9	8	ns	12.7	10	10.6	ns
51	Propanoic acid	7.2 b	0.5 a	8.0 c	***	1	6	ns	12 c	nd a	9.0 b	***
52	Isobutanoic acid	0.09 b	0.10 b	0.06 a	*	0.18	0.2	ns	0.07 b	0.05 a	0.06 ab	*
53	Butanoic acid	0.07	0.04	0.08	ns	0.05 a	0.3 b	*	0.10	0.04	0.04	ns
54	2-Methylbutanoic acid	2.5 c	1.3 b	0.7 a	***	0.75 a	1.2 b	**	1.7 c	1.2 b	0.6 a	***

55	Hexanoic acid	0.51 c	0.39 b	0.30 a	***	0.3	0.33	ns	0.39 a	1.6 b	0.58 a	***
56	Heptanoic acid	0.08 b	0.06 a	0.06 a	**	0.01 a	0.05 b	***	0.03 a	0.03 a	0.06 b	***
57	(E)-3-Hexenoic acid	0.09 c	0.06 b	0.04 a	***	tr a	0.01b	**	0.04 b	0.03 a	0.06 c	***
58	Octanoic acid	0.14	0.15	0.14	ns	0.3	0.13	ns	0.12 a	2.0 b	0.13 a	***
59	Decanoic acid	0.02	0.03	0.03	ns	0.02	nd	ns	nd a	0.2 b	0.01 a	***
60	Benzoic acid	0.08	0.07	0.07	ns	0.10	0.3	ns	0.16	0.16	0.20	ns
	Aldehydes											
61	Pentanal	0.14 a	0.38 b	0.14 a	***	0.5	0.4	ns	0.17 b	0.3 c	nd a	**
62	Heptanal	0.01	0.02	0.02	ns	nd	nd		tr b	tr b	nd a	***
63	Octanal	0.04	0.08	0.06	ns	0.06	0.06	ns	0.02	0.04	0.02	ns
64	2-Heptenal	0.2	0.22	nd	ns	0.3	0.7	ns	nd a	0.2 b	nd a	***
65	Nonanal	0.05	0.04	0.05	ns	0.05	nd	ns	0.03 b	nd a	tr a	***
66	(E)-2-Octenal	0.03	0.04	0.02	ns	0.03	0.05	ns	tr b	0.02 c	nd a	**
67	Benzaldehyde	0.53 a	0.67 b	0.7 b	***	0.9 b	0.7 a	**	0.70 a	1.2 b	0.93 ab	**
68	(E)- 2-Decenal	0.18	0.3	0.3	ns	0.12	0.10	ns	0.03	nd	0.04	ns
	Terpenes/ terpenoids											

69	Limonene	0.02	0.02	0.02	ns	0.07 b	nd a	***	0.08 a	0.8 b	0.01 a	***
70	6-Methyl-5-hepten-2-one	0.09 a	0.17 b	0.10 a	***	0.12	0.14	ns	0.07 a	0.19 b	0.08 a	***
71	Linalool oxide	0.04 a	0.08 b	0.05 a	***	0.19	0.14	ns	0.04 b	nd a	0.13 a	***
72	(+)-Cycloisosativene	nd a	0.02 b	nd a	***	0.10	0.14	ns	nd	nd	nd	
73	Copaene	0.08 a	0.37 c	0.14 b	***	1.8 b	0.4 a	*	0.8 c	0.3 a	0.6 b	***
74	Dihydroedulan	1.0 c	0.1 a	0.4 b	***	0.01	tr	ns	1.8 c	0.10 a	1.02 b	***
75	Linalool	0.24	0.5	0.30	ns	0.08	0.05	ns	0.16 a	0.21 b	0.25 c	***
76	Caryophyllene	nd a	tr b	nd a	***	0.02	tr	ns	0.02 c	nd a	tr b	***
77	α-Terpineol	0.22 b	0.22 b	0.16 a	***	1.0	0.8	ns	0.28 a	0.6 b	0.25 a	***
78	α-Muurolene	0.01 a	0.05 c	0.02 b	***	0.3 b	0.03 a	**	0.12 c	0.06 a	0.09 b	***
79	β-Damascenone	0.21 a	0.31 b	0.21 a	***	0.10	0.11	ns	0.20 a	0.30 b	0.22 a	***
80	Isogeraniol	0.05 a	0.07 c	0.06 b	**	0.07	0.06	ns	0.05 a	0.09 c	0.06 b	***
81	Geraniol	0.16 a	0.38 c	0.23 b	***	0.3	0.14	ns	0.3 a	0.7 b	0.27 a	***
82	Iridomyrmecin	0.06	0.07	0.03	ns	0.03 a	0.08 b	**	0.03	0.03	0.03	ns
	Phenolic compounds											
83	p-Creosol	27a	37 c	32 b	***	26	26	ns	30 b	2 a	29 b	***

84	Phenol	0.09 b	0.05 a	0.09 b	**	0.06 a	0.2 b	*	1	1	0.22	ns
85	p-Ethyl guaiacol	0.22 c	0.19 b	0.14 a	***	0.16	0.17	ns	0.11	0.11	0.13	ns
86	p-Cresol	0.5 c	0.14 a	0.3 b	***	0.07	0.3	ns	0.77 b	0.11 a	0.79 b	***
87	p-Propyl guaiacol	0.17 b	0.09 a	0.09 a	**	0.04	0.04	ns	0.08 b	0.03 a	0.10 c	***
88	Eugenol	0.08	0.07	0.07	ns	0.03	0.03	ns	0.02 a	0.02 a	0.03 b	**
89	4-Ethyl phenol	1.9 b	1.2 a	1.0 a	***	1.6	1.9	ns	5	2	2.5	ns
90	Vanillin	0.06 a	0.12 b	0.05 a	**	0.14 b	0.04 a	*	0.14 b	0.15 b	0.10 a	**
91	Tyrosol	1.0	0.3	0.6	ns	0.16	0.4	ns	0.6	0.4	0.5	ns
	Hydrocarbons											
92	Octane	0.4 a	0.9 b	1.1 b	***	0.1	0.4	ns	0.06 a	0.2 b	0.11 ab	*
93	Decane	0.8 b	0.49 a	0.5 a	***	0.46 b	0.23 a	**	0.3	0.25	0.3	ns
94	o-Xylene	0.03 a	0.04 b	0.02 a	**	0.03	0.02	ns	0.02 a	0.03 a	1.1 b	***
95	Styrene	0.03 a	0.02 a	0.2 b	*	0.3	0.02	ns	0.03 a	0.4 b	0.06 a	**
96	2-Bornene	1.0 a	1.6 b	0.87 a	***	0.5	0.5	ns	0.7 a	1.0 c	0.9 b	***
	Other compounds											
97	Dimethyl sulfide	1.0 b	0.7 a	0.5 a	**	0.7	1.4	ns	0.2 a	0.5 b	0.26 a	***

98	Theaspirane A	0.6	0.8	0.6	ns	0.11	0.18	ns	1.1 b	1.1 b	0.8 a	*
99	Theaspirane B	0.7	0.8	0.7	ns	0.12	0.18	ns	1.2 b	1.3 b	0.8 a	*
100	Dimethyl sulfoxide	0.10 c	0.08 b	0.05 a	***	0.07 a	0.7 b	**	0.09 b	0.11 b	0.06 a	**
101	Butyrolactone	0.04 b	0.06 c	nd a	***	0.05 a	0.6 b	**	0.2 b	0.10 a	0.13 ab	*
102	1,4-Dimethoxybenzene	nd	nd	nd		nd	nd		0.05	0.05	0.04	ns

[a] Mean values for 2 fermenters, each analyzed in triplicate (n = 6). For each cultivar, data in the same row with different letters are significantly different ($p < 0.05$); tr, <0.01; nd, not detected (a zero was used as the concentration value in place of the not detected entry).

[b] Probability, as obtained from ANOVA, that there is a difference between means: * significant at the 5% level; ** significant al the 1% level; *** significant at 0.1% level; ns, no significant difference between means ($P > 0.05$).